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我国小鲵科一新属新种的描述*

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关键词 两栖纲，有尾目 小鲵科 肥鲵属新属 商城肥鲵新种 河南省

1959年至1981年，在大别山、桐柏山、伏牛山及太行山等地区调查，发现小鲵科一种，外部形态和头骨特征与已知小鲵科各属均有明显区别。现将商城标本与相近的小鲵属和北鲵属的主要特征对比如表1（图1—3）。

从表1可以看出，商城标本与相近的小鲵属和北鲵属都有显著区别，应为一个新属新种。模式标本保存在新乡师范学院。现描述如下：

表1 肥鲵属与相近属的特征比较

性 状		小鲵属 *Hynobius*	肥鲵属 *Pachyhynobius*	北鲵属 *Ranodon*
犁骨齿列	形 状	"V"形，外枝显著短于内枝	"V"形，外枝略短于内枝	"八"形，无内枝
	内侧端起于犁腭骨位置	后端	后端	中部或后1/3处
头骨形状		多为前后几等宽	前窄后宽	前窄后宽
囟 门		无	无	有
上颌骨与翼骨		不连，相距远	相连	不连，相距远
鳞 骨		内侧不隆起	内侧显著隆起	内侧不隆起
肺		长	短小	短小
四 肢		适中	短弱	适中
唇 褶		无	有	有

肥鲵属 新属 *Pachyhynobius* gen nov.（图3—4）

属征 头骨前窄后宽，上颌骨间距远小于方骨外侧间距；上颌骨与翼骨相连接；鳞骨内侧显著隆起；犁骨齿列呈"V"形，近内鼻孔后内侧；无囟门；泪骨入眼眶，不入外鼻孔。体形明显肥壮，尾短于头体长；有唇褶，较弱；前后肢短弱，指4，趾5。

*本文承胡淑琴教授审阅，并提出修改意见。先后参考野外工作的同志有：瞿文元、王才安、夏祥云、卢钦旭、刘靖红等。王宜生同志绘图。谨此一并致谢。
本文1984年12月22日收到。

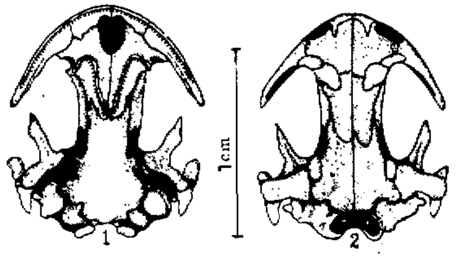


图 1 中国小鲵*Hynobius chinensis* 58302号浙江标本
1.示腹面 2.示背面

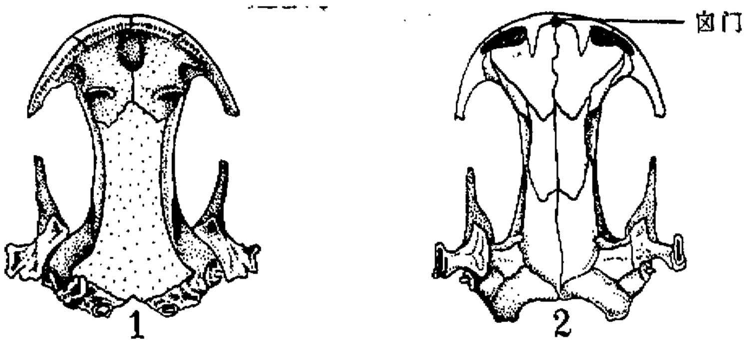


图 2 新疆北鲵*Ranodon sibiricus*
头骨（录自Chang 1936）1.示腹面 2.示背面

模式种：商城肥鲵 新种 *Pachyhynobius shangchengensis* sp. nov.

新 种 描 述

商城肥鲵 新种 *Pachyhynobius shangchengensis* sp. nov.（图3—4）

模式标本 正模♂（00227），配模♀（00223），河南商城黄柏山，海拔780米，1981年5月13日，瞿文元采。副模7♂♂、9♀♀、幼体13；1959年8月采自商城金岗台，海拔380米，1981年5月采自商城黄柏山，海拔780米。

形态描述 体形肥壮，雄鲵平均体全长167.3毫米左右，雌鲵164.3毫米左右。从吻至头顶部显然逐渐高起；躯干浑圆；尾基部粗壮，略呈方形，往后渐侧扁，末端钝。头长大于头宽，吻钝圆，吻端略突出下唇；唇褶较弱；鼻孔近吻端，鼻间距与眼间距几相等，或略大于眼间距；口裂位于眼后下方；颈褶发达；上下颌具钩状细齿；犁骨齿两短列，每侧有12枚左右，“\/”形，位于犁腭骨后缘，近内鼻孔后内侧，左右两齿列间间距约0.6—0.8毫米。

表 2　　商城肥鲵 *Pachyhynobius shangchengensis* 河南商城

	正模 ♂ 00227	7♂♂	配模 ♀ 00223	9♀♀	幼体 13个
全长	165.0	150.0—184.0 167.3	167.0	157.0—176.0 164.3	72.0—83.0 77.5
头体长	96.0	91.0—114.4 102.3	104.0	95.0—106.0 100.8	42.2—48.0 44.6
头长	23.8 24.8%	20.1—27.0 22.8 22.0%	22.5 21.6%	20.0—22.8 22.1 21.9%	12.0—14.4 13.4 30.0%
头宽	20.6 21.5%	16.0—21.5 19.5 19.0%	16.8 16.2%	15.0—18.9 17.4 17.2%	
头高	15.7 16.4%	8.4—15.7 13.5 13.1%	11.7 11.3%	10.8—14.5 12.2 12.1%	9.0—11.3 10.0 22.4%
眼间距	7.5 7.8%	5.7—7.5 6.9 6.0%	5.8 5.6%	5.0—6.3 5.6 5.5%	
眼径	5.0 5.2%	4.0—5.6 4.9 4.7%	4.9 4.7%	4.0—5.3 4.7 4.6%	
尾长	69.0 71.9%	59.0—69.6 64.9 63.4%	63.0 60.6%	58.0—70.0 63.6 63.0%	31.2—35.5 33.0 74.0%
尾基宽	13.5 14.1%	10.9—14.5 13.6 13.2%	13.4 12.9%	12.0—14.5 13.2 13.0%	
尾高	14.2 14.8%	10.5—15.0 13.1 12.8%	14.3 13.8%	13.6—17.8 15.1 14.9%	7.1—11.7 9.5 21.3%
前肢长	19.5 20.3%	16.4—19.5 18.3 17.8%	17.4 16.7%	15.7—19.9 17.6 17.4%	10.0—11.0 10.1 22.6%
后肢长	21.8 22.7%	20.0—24.5 22.3 21.7%	20.4 19.6%	19.5—24.4 21.3 21.1%	10.0—11.5 10.6 23.8%
腋至胯距	49.3 51.4%	45.7—57.0 52.7 51.5%	56.1 53.9%	49.0—56.1 52.0 51.5%	

注：量度以毫米为单位，百分率是各部量度与头体长之比。

四肢短弱，前肢尤甚，前肢向前伸时，指末端距眼甚远，约为前肢基部到吻端间距之半，前后肢贴体相对时，指趾端相距3—5个肋沟，指四，长序为3、2、4、1，趾五，长序为3、4、2、5、1，指、趾较扁；掌、蹠部无角质鞘。

尾长短于头体长；尾背鳍褶始于后肢后伸的末端部位，腹鳍褶始于尾长的后1/2处；肛部微隆起，肛孔纵裂。

皮肤光滑，头顶有不明显的"V"形嵴；眼后有一细纵沟达颈褶；头后至背尾鳍褶起始处有一浅脊沟，肋沟13条。

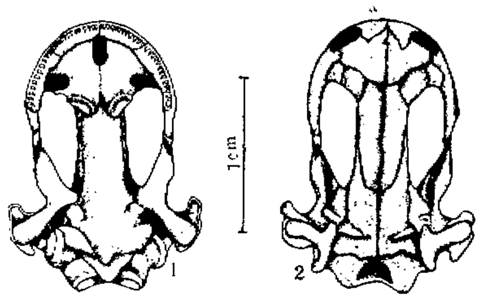


图3 商城肥鲵 新种 *Pachyhynobius shangchengensis* sp. nov.
00226号 ♀ 头骨 1.示腹面 3.示背面

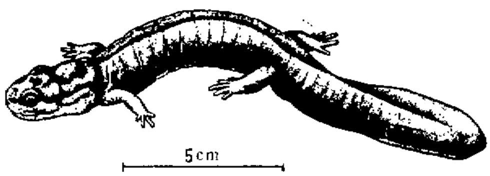


图4 商城肥鲵 新种 *Pachyhynobius shangchengensis*, sp. nov
00224号 ♀ 背侧面观

头骨：左右上颌骨之间距窄；翼骨较发达与上颌骨相连接；鳞骨内侧明显隆起，左右额骨和顶骨之中缝处有稜嵴。

生活时体背面深褐色，体侧色稍浅，腹面灰褐色或灰白色，刚变态的亚成体在体侧和尾侧有分散小白点。甲醛浸泡时间长的标本，体色为黄褐色。

第二性征　液浸标本，雄鲵肛裂前部灰蓝色，后部色浅；雄鲵肛裂前部与体腹面色同，肛裂后部色浅。

幼体　5月中旬采到的幼体，平均体全长77.5毫米时，头体长44.6毫米，尾长为全长的2/5，前肢长10.1毫米，后肢长10.6毫米，指趾末端角质化，似爪状。头部宽扁，渐向吻端倾斜，体背较高而窄，向两侧渐宽，尾较侧扁。吻钝圆，上唇突出于下唇，两侧唇褶较显；眼位于头背侧，外鳃三对，第一对短，第三对长，平均长度3.4毫米。颈褶后缘游离，肋沟明显；尾肌较弱，鳍褶薄而发达，背鳍褶前达体背中部，其长

度约与头体长相等，腹鳍褶达肛裂后缘，末端尖细。背面深褐色，有的浅褐，满布不规则的斑纹，头背斑纹细密，体、尾背侧及尾鳍褶的斑纹较大，腹面白色。

生活习性　5—8 月见于海拔380—780米的山沟溪流内，常栖于流速缓慢，清澈见底的大小水坑的水底或水底的石面上，坑底多为沙石。受惊后常迅速钻入石下或石缝中，有时也到水坑的上段流水内觅食。

参考文献

刘承钊、胡淑琴、杨抚华 1960 四川巫山两栖动物初步调查报告。动物学报12（2）:278—292

胡淑琴、赵尔宓、刘承钊 1966 秦岭及大巴山地区两栖爬行动物调查报告。动物学报18（1）:57—89

费梁、叶昌媛 1983 小鲵科的分类探讨，包括一新属。两栖爬行动物学报 2（4）:31—37

佐藤井岐雄 1943 日本产有尾类总说。日本出版社. 1—520

Dunn E. R. 1923 The salamanders of the family Hynobiidae. *Proc. Amer. Acad.* Boston. 58 (13): 445—523

Chang M. L. Y. 1936 Contribution a l'etude morphologique, biologique *et* systematique des amphibiens urodeles de la Chine. Picart, Paris. 156pp.

Thorn R. 1968 Les salamandres d'Europe, d'Asie *et* d' Afrique du Nord. Paul Lechevalier, Paris. 1—376

DESCRIPTION OF A NEW GENUS AND SPECIES OF HYNOBIIDAE OF CHINA

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Herpetological surveys were made from 1959 to 1981 in the Dabie, Tongbai, Funiu, and Taihang Mountains in Henan Province, and a new genus and species was discovered.

Pachyhynobius gen. nov. (Figs. 3, 4)

Diagnosis: This genus is characterized by, 1) the anterior part of the skull narrower than the posterior, with the distance between the posterior tips of the maxillae much shorter than between the outer borders of the quadrates; 2) maxilla connected with pterygoid; 3) the inner side of the squamosum much protruded; 4) vomerine teeth series "\/"-shaped; 5) premaxillary fontanelle absent; 6) lachrymal bone entering the orbit but not external nares; 7) body rather stout, tail shorter than head-body, and limbs rather short and weak; 8) labial folds present; 9) fingers 4 and toes 5.

Type species: *Pachyhynobius shangchengensis* sp. nov. *Pachyhynobius shangchengensis* sp. nov.

Holotype: No. 00227, adult male, Huangbaishan, Shangcheng County, Henan, altitude 780m, collected on May 13, 1981 by Qu Wenyuan.

Allotype: No. 00223, adult female, collected with the holotype.

Paratypes: 7♂♂, 9♀♀, and 13 larvae, collected in August, 1959 and May 1981 from Shangcheng, altitude 380—780m.

Type specimens are kept in the Department of Biology, Xinxiang Normal College, Henan.

Key words: Amphibia, Caudata Hynobiidae *Pachyhynobius* gen. nov *Pachyhynobius shangchengensis*, sp. nov. Henan Province

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On the nucleoli of the dinoflagellate *Prorocentrum*

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Abstract

To date no nucleolus had been observed in *Prorocentrum* under the light microscope. The author failed to show the nucleoli of *P. micans* and *P. cassubica* with eosin in 70% alc or with methyl green-pyronin. But when these dinoflagellates were treated with an Ag-1 technique which had been improved for demonstrating NORs in unicellular organisms, nucleoli were stained dark brown or black, while all other parts showed no colour. When the materials were stained well, only the central part of the nucleolus was stained. Under the electron microscope, it was observed that all the silver grains were concentrated in the pars fibrosa of the nucleolus. *P. cassubica* had only one small oblate nucleolus attached to the nuclear envelope, with NOR usually in the shape of the letters O or C. *P. micans* had 1—7 nucleoli of various sizes and shapes with NORs in various complicated forms. The number of nucleoli bore a certain relationship to the living state of the dinoflagellate. One day after fresh medium was added, cells with 3 nucleoli were most common, and 28.5% of the individuals had 4—6 nucleoli. Cells having only one nucleolus accounted for 8.6%. 3 days after, cells with 2 nucleoli became dominant, and those with 4—6 decreased to 18.4%. After a month, cells with 1 nucleolus became most abundant, cells having 4 nucleoli decreased to 2.4%, and no cells had 5 or 6 nucleoli.

Key words: Nucleolus dinoflagellate silver-staining culture

Introduction

It has been reported that no nucleolus occurs in several dinoflagellates, especially in species belonging to *Prorocentrum* and *Exuviella* (Dodge, 1966).

This is surprising. Although nucleoli were observed afterwards in *Prorocentrum micans* under electron microscope (Zingmark, 1970), it remains difficult to get a complete picture of nucleoli for study by ultrathin sections. To investigate the variation in nucleolus number, the present author improved the Ag-1 procedure for demonstrating nucleolar organizer regions(NORs) in unicellular organisms under both light and electron microscopes (Li Jing-yan, 1981). In the present work, the author used this improved technique to demonstrate nucleoli in *Prorocentrum micans* and in *P. cassubica*. For the first time, the morphology of these nucleoli could thus be studied under microscope and their numbers counted. *Prorocentrum micans* is an organism which may cause red tides. The investigation of their nucleoli may be of help to predict these.

Materials and methods

Prorocentrum micans (LB1136) obtained from the Culture Centre of Algae and Protozoa (Cambridge, England) and *P. cassubica* (LB1596) obtained from The Culture Collection of Algae at the University of Texas were grown in AE_{50} medium at 20°C.

Absolute alcohol, 10% formalin, Carnoy's fluid (3 : 1), and 2% glutaraldehyde (phosphate buffer, pH 7.4) were used as fixatives. Part of the fixed material, after washing, was used for preparing smears. Three staining methods were used: 1. 0.5% eosin in 70% alcohol, 2. Unna's methyl green -pyronin, 3. the improved Ag-1 technique (Li Jing-yan, 1981).

All the steps in the improved Ag-1 technique were carried out in centrifuge tubes. Material fixed with alcohol, formalin, or glutaraldehyde should be treated with Carnoy's fluid (3 : 1) for 5 to 10 min. For microscopic observation, after thorough washing with redistilled water, materials were stained with freshly prepared 50% $AgNO_3$ containing 1 % acetic acid, at 37°C for 6 h. For electron microscope observation, staining for 2 or 3 hours proved sufficient. The silver stained materials were washed thoroughly with redistilled water and were then smeared or embedded in Epon 812

Because of the high specificity of this improved Ag—1 procedure, only nucleoli were stained, and all other cell components, including the condensed dinoflagellate chromosomes, had no colour at all. So, the contour of nuclei could not be distinguished. Several smears were counterstained with methyl green to show chromosomes and the edge of nuclei. Part of the fixed material, after washing, was immersed into 0.2% boric acid solution for a few minutes before silver staining. This boric acid treatment would make the condensed

chromosomes pale yellow after silver staining, while the nucleoli were still stained dark.

The materials embedded in Epon 812 were ultrathin-sectioned and stained with uranium acetate and lead citrate.

Observations and discussion

In common histological preparations, cytoplasm and nucleoli in various cells and tissues are always stained rose-pink by eosin alcoholic solution. But when *P. micans* and *P. cassubica* were stained with eosin solution for a long time, there were no nucleolus-like structures demonstrated in nuclei, while cytoplasm was stained rose-pink clearly. The condensed dinofIgellate chromosomes were not stained either.

It could be inferred that the RNA-rich nucleoli would be stained bright red while chromosomes blue-green in the preparations stained with methyl green-pyronin. But this was inconsistent with the cases of *P. micans* and *P. cassubica*. The fact was that the cytoplasm was stained red and chromosomes blue-green, while there were no structures stained bright red in the nuclei of *Prorocentrum*. When the preparations were carefully examined under oil-immersion, sometimes a few small areas stained pale red or pale purple were seen in the nuclei. It was likely that these areas were nucleoli but they were too pale and indistinct to be distinguished from the pale red nucleoplasm around the chromosomes, and only on very few occasions could they be identified as nucleoli. So, it was impossible to investigate the morphology of these dinoflagellates' nucleoli and count their numbers with this staining method.

When dinoflagellates were treated with the improved Ag—1 procedure, the nucleoli of both *Prorocentrum* species were stained very distinctly. They were dark brown or deep black, while all the other components in the cell showed no colour at all.

If the cells were not over-stained and the preparations carefully examined, it could be seen that only the central part of the nucleolus was stained and the peripheral region remained colourless (when the cells were over-stained the whole nucleolus was stained) .

Under the eletron microscope (the cells were stained for only 2 or 3 h), it was observed that all the siver grains were concentrated in the central pars fibrosa, and there were no silver grains in the peripheral pars granulosa nor in the condensed chromatin cords within the dinoflagellate nucleolus.

It is known that the silver staining demonstrating NORs may possess very high specificity and there is only one species of protein shown by this silver



Explanation of plate

Dinoflagellates stained with the improved Ag—1 technique. Materials had been treated with boric acid before staining with $AgNO_3$ in fig. 1. Materials were counterstained with methyl green in fig. 4, 7 and 8.

Fig. 1—3. *Prorocentrum cassubica.*
Fig. 1. Nucleolus attached to the nuclear envelope.
Fig. 2. and 3. O-shaped NOR.
Fig. 4—8. *Prorocentrum micans.*
Fig. 4. The individual with 2 nucleoli.
Fig. 5. The individual with 4 nucleoli.
Fig. 6. The individual with 5 nucleoli.
Fig. 7. The individual with 7 nucleoli.
Fig. 8. The NOR like a spiral line.

staining. This is an acidic protein participating in the transcription activity on pre-rRNA genes (Hubell *et al.*, 1979). So, the active NOR in dinoflagellate nucleolus is just the pars fibrosa.

The nucleus of *P. cassubica* possesses only one small nucleolus which is oblate and attached to the nuclear envelope. In rare occasions, the nucleolus was located within a nuclear bud extruding into the cytoplasm. This would give the false impression that the nucleolus had departed from the nucleus. It was observed that the NOR in the nucleolus of *P. cassubica* was usually ringlike or C-shaped.

The morphology of the nucleoli of *P. micans* is greatly different from that of *P. cassubica*. Its number of nucleoli in the nucleus is variable. In different individuals, and even in the same nucleus, these nucleoli may vary in size and shape. This is in striking contrast with the nucleolus in *P. cassubica*. Very big nucleoli could be found be found only in nuclei which had one or two nucleoli. The total voume of the nucleoli in one nucleus is not a constant, but varies strongly.

The NORs of *P. micans* take various shapes. They usually look like complicated wound cords, but sometimes they form hollow spheres with thick walls. Occasionally they even appear as long spiral lines in rod-like nucleoli.

Most of the individuals possess 1 to 6 nucleoli, and in very rare cases there are 7. It seems that there is a certain relationship between the number of nucleoli in a nucleus and the physiological state of the dinoflagellate. One day after fresh culture medium was added to the old medium (with about equal volumes), the individuals having 3 nucleoli were the most common (33.3% of all individuals); 28.5% of the individuals had 4 to 6 nucleoli; the individuals with only one nucleolus were at 8.6%. Three days after fresh medium was added, individuals with 2 nucleoli became most numerous (38.8%), cells with 4 to 6 nucleoli decreased to 18.4%, and individuals possessing only 1 nucleolus increased to 13.9%. One month after, the elongate nuclei became spherical, the individuals possessing 1 nucleolus were the most common (36.6%), the

Table 1　The variation of nucleolus number in nucleus of *Prorocentrum micans* with the ageing of culture medium.

Days after adding fresh medium	Individuals with different number of nucleoli				
	1	2	3	4	5 — 6
1 day	8.6%	28.6%	33.3%	21.4%	7.1%
3 days	13.9%	38.8%	28.9%	14.5%	3.9%
30days	36.6%	31.5%	29.5%	2.4%	0 %

cells with 4 nucleoli were only at 2.4%, and there were no individuals with 5 or 6 nucleoli (Table. 1).

There are two hypotheses to explain why the number of the nucleoli decreases with increasing age of the culture medium. One is that two or more nucleoli fuse into a larger one. But this seems to be inconsistent with the facts, for the individuals living in the aged culture medium usually possess small nucleoli only, and sometimes very small ones. Another explanation is that the decrease is caused by a reduction in protein synthesis within the cells when the conditions become worse. When the biosynthesis of protein decreases, the requirement for the newly formed ribosomal subunits decreases too. As a result more and more nucleolar organizers become inactive and take no part in the construction of the nucleolus.

P. micans is one of the dinoflagellates which may cause red tides. The nucleoli number in these dinoflagellates' nuclei may reflect, at least to a certain extent, their condition. Perhaps examining the nucleoli of these dinoflagellates in the sea might contribute to forecasting red tides.

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References

Dodge, J. D., 1966. The Dinophyceae. In The chromosomes of the algae. Arnold, p. 96—115

Hubbell, H. R., L. I. Rothblum & T. C. Hsu, 1979, Identification of a siver binding protein associated with the cytological silver staining of actively transcribing nucleolar regions. Cell Biol. Int. Rep. 3 :615—622

Li Jing-yan, 1981. A cytochemical silver staining method suitable for demonstrating nucleolar organizer regions in unicellular eukaryotes under light microscope and electron microscope. Symp. microsc. & submicrosc. Morphol., Shijiazhuang, 1981

Zingmark, R. G., 1970. Ultrastructural studies on two kinds of mesocaryotic dinoflagellate nuclei. Am. J. Bot. 57:586—592